

FILE 'HOME' ENTERED AT 11:39:14 ON 05 FEB 2008

=> b reg

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.21	0.21

FILE 'REGISTRY' ENTERED AT 11:39:29 ON 05 FEB 2008

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STRUCTURE FILE UPDATES: 4 FEB 2008 HIGHEST RN 1001463-85-9

DICTIONARY FILE UPDATES: 4 FEB 2008 HIGHEST RN 1001463-85-9

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<http://www.cas.org/support/stngen/stndoc/properties.html>

=> S 107-13-1/RN

L1 1 107-13-1/RN

=> S 79-06-1/RN

L2 1 79-06-1/RN

=> index bioscience

FILE 'DRUGMONOG' ACCESS NOT AUTHORIZED

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.46	0.67

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS, CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB, DRUGMONOG2, DRUGU, EMBAL, EMBASE, ...' ENTERED AT 11:40:16 ON 05 FEB 2008

69 FILES IN THE FILE LIST IN STINDEX

Enter SET DETAIL ON to see search term postings or to view search error messages that display as 0\* with SET DETAIL OFF.

=> b reg

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.65	1.32

FILE 'REGISTRY' ENTERED AT 11:40:35 ON 05 FEB 2008

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DICTIONARY FILE UPDATES: 4 FEB 2008 HIGHEST RN 1001463-85-9

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REGISTRY includes numerically searchable data for experimental and  
predicted properties as well as tags indicating availability of  
experimental property data in the original document. For information  
on property searching in REGISTRY, refer to:

<http://www.cas.org/support/stngen/stndoc/properties.html>

=> sel L1 chem  
E1 THROUGH E17 ASSIGNED

=> sel L2 chem  
E18 THROUGH E26 ASSIGNED

=> index bioscience  
FILE 'DRUGMONOG' ACCESS NOT AUTHORIZED  
COST IN U.S. DOLLARS

SINCE FILE	TOTAL
ENTRY	SESSION
1.18	2.50

FULL ESTIMATED COST

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE,  
AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS,  
CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB,  
DRUGMONOG2, DRUGU, EMBAL, EMBASE, ...' ENTERED AT 11:40:50 ON 05 FEB 2008

69 FILES IN THE FILE LIST IN STINDEX

Enter SET DETAIL ON to see search term postings or to view  
search error messages that display as 0\* with SET DETAIL OFF.

=> s e1-17 (s) e18-26  
16 FILE AGRICOLA  
9 FILE ANABSTR  
19 FILE ANTE  
5 FILE AQUALINE  
2 FILE AQUASCI  
42 FILE BIOENG  
107 FILE BIOSIS  
143 FILE BIOTECHABS  
143 FILE BIOTECHDS  
29 FILE BIOTECHNO  
16 FILE CABA  
3865 FILE CAPLUS  
15 FILES SEARCHED...  
64 FILE CEABA-VTB  
30 FILE CIN

```

4 FILE CONFSCI
2 FILE CROPU
3 FILE DDFB
4 FILE DDFU
51 FILE DGENE
10 FILE DISSABS
3 FILE DRUGB
4 FILE DRUGU
1 FILE EMBAL
64 FILE EMBASE
43 FILE ESBIOBASE
30 FILES SEARCHED...
7 FILE FROSTI
14 FILE FSTA
6 FILE HEALSAFE
3317 FILE IFIPAT
57 FILE LIFESCI
53 FILE MEDLINE
45 FILE NTIS
291 FILE PASCAL
47 FILES SEARCHED...
2 FILE PHIN
191 FILE PROMT
43 FILE RDISCLOSURE
306 FILE SCISEARCH
1 FILE SYNTHLINE
240 FILE TOXCENTER
72 FILE USGENE
22634 FILE USPATFULL
3144 FILE USPATOLD
62 FILES SEARCHED...
2942 FILE USPAT2
10 FILE WATER
4551 FILE WPIDS
28 FILE WPIFV
4551 FILE WPINDEX

47 FILES HAVE ONE OR MORE ANSWERS, 69 FILES SEARCHED IN STINDEX

L3 QUE (ACRYLON/BI OR ACRYLONITRILE/BI OR CARBACRYL/BI OR CYANOETHENE/BI OR C
YANOETHYLENE/BI OR FUMIGRAIN/BI OR "NSC 6362"/BI OR PROPENENITRILE/BI
OR VCN/BI OR VENTOX/BI OR "VINYL CYANIDE"/BI OR 107-13-1/BI OR 2-PROPE
NENITRILE/BI OR 29754-21-0/BI OR 63908-52-1/BI OR 769126-92-3/BI OR 76
9134-66-9/BI) (S) (ACRYLAMIDE/BI OR "ACRYLIC AMIDE"/BI OR "BIO-ACRYLAM
IDE 50"/BI OR ETHYLENECARBOXYAMIDE/BI OR "NSC 7785"/BI OR PROPENAMIDE/B
I OR "VINYL AMIDE"/BI OR 2-PROPENAMIDE/BI OR 79-06-1/BI)

=> s L3 (s) enzym#####
2 FILE AGRICOLA
1 FILE ANABSTR
2 FILE AQUASCI
19 FILE BIOENG
11 FILE BIOSIS
65 FILE BIOTECHABS
65 FILE BIOTECHDS
12 FILE BIOTECHNO
1 FILE CABA
14 FILES SEARCHED...
47 FILE CAPLUS
12 FILE CEABA-VTB
2 FILE CIN

```

30 FILE DGENE  
5 FILE EMBASE  
11 FILE ESBIOBASE

30 FILES SEARCHED...

1 FILE FROSTI  
8 FILE FSTA  
1 FILE HEALSAFE  
16 FILE IFIPAT  
24 FILE LIFESCI  
3 FILE MEDLINE  
13 FILE PASCAL

47 FILES SEARCHED...

8 FILE PROMT  
1 FILE RDISCLOSURE  
7 FILE SCISEARCH  
3 FILE TOXCENTER  
72 FILE USGENE  
125 FILE USPATFULL  
5 FILE USPATOLD  
13 FILE USPAT2  
1 FILE WATER  
54 FILE WPIFS  
1 FILE WPIFV  
54 FILE WPINDEX

34 FILES HAVE ONE OR MORE ANSWERS, 69 FILES SEARCHED IN STINDEX

L4 QUE L3 (S) ENZYM#####

=> s L4 (s) (convert### or produc### or generat###)

1 FILE ANABSTR  
13 FILE BIOENG  
5 FILE BIOSIS  
45 FILE BIOTECHABS

11 FILES SEARCHED...

45 FILE BIOTECHDS  
4 FILE BIOTECHNO  
1 FILE CABA  
15 FILE CAPLUS  
5 FILE CEABA-VTB

17 FILES SEARCHED...

27 FILE DGENE

23 FILES SEARCHED...

1 FILE EMBASE  
5 FILE ESBIOBASE

30 FILES SEARCHED...

1 FILE FROSTI  
1 FILE FSTA  
11 FILE IFIPAT

39 FILES SEARCHED...

14 FILE LIFESCI  
7 FILE PASCAL

47 FILES SEARCHED...

4 FILE PROMT  
1 FILE RDISCLOSURE  
2 FILE SCISEARCH

59 FILES SEARCHED...

72 FILE USGENE  
75 FILE USPATFULL  
3 FILE USPATOLD  
10 FILE USPAT2

```

63 FILES SEARCHED...
    15 FILE WPIDS
    15 FILE WPINDEX

```

26 FILES HAVE ONE OR MORE ANSWERS, 69 FILES SEARCHED IN STINDEX

L5 QUE L4 (S) (CONVERT### OR PRODUC### OR GENERAT###)

=> s L5 and (detect### or determin### or measur### or quantitat###)

```

    1 FILE ANABSTR
    1 FILE BIOENG
10 FILES SEARCHED...
    9 FILE BIOTECHABS
    9 FILE BIOTECHDS
    1 FILE BIOTECHNO

```

```

13 FILES SEARCHED...
    2 FILE CAPLUS
    20 FILE DGENE

```

```

23 FILES SEARCHED...
    1 FILE ESBIOBASE

```

```

30 FILES SEARCHED...
41 FILES SEARCHED...
    1 FILE LIFESCI
    1 FILE PASCAL

```

```

47 FILES SEARCHED...
59 FILES SEARCHED...
    72 FILE USPATFULL
    3 FILE USPATOLD
    9 FILE USPAT2

```

```

63 FILES SEARCHED...
    6 FILE WPIDS
    6 FILE WPINDEX

```

15 FILES HAVE ONE OR MORE ANSWERS, 69 FILES SEARCHED IN STINDEX

L6 QUE L5 AND (DETECT### OR DETERMIN### OR MEASUR### OR QUANTITAT###)

=> d rank

```

F1      72  USPATFULL
F2      20  DGENE
F3       9  BIOTECHABS
F4       9  BIOTECHDS
F5       9  USPAT2
F6       6  WPIDS
F7       6  WPINDEX
F8       3  USPATOLD
F9       2  CAPLUS
F10      1  ANABSTR
F11      1  BIOENG
F12      1  BIOTECHNO
F13      1  ESBIOBASE
F14      1  LIFESCI
F15      1  PASCAL

```

=> fil f2-14

COST IN U.S. DOLLARS

SINCE FILE	TOTAL
ENTRY	SESSION
13.65	16.15

FULL ESTIMATED COST

FILE 'DGENE' ENTERED AT 11:53:34 ON 05 FEB 2008

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FILE 'WPIDS' ENTERED AT 11:53:34 ON 05 FEB 2008  
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FILE 'WPINDEX' ACCESS NOT AUTHORIZED

FILE 'USPATOLD' ENTERED AT 11:53:34 ON 05 FEB 2008  
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FILE 'CAPLUS' ENTERED AT 11:53:34 ON 05 FEB 2008  
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COPYRIGHT (c) 2008 THE ROYAL SOCIETY OF CHEMISTRY (RSC)

FILE 'BIOENG' ENTERED AT 11:53:34 ON 05 FEB 2008  
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FILE 'BIOTECHNO' ENTERED AT 11:53:34 ON 05 FEB 2008  
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FILE 'ESBIOBASE' ENTERED AT 11:53:34 ON 05 FEB 2008  
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FILE 'LIFESCI' ENTERED AT 11:53:34 ON 05 FEB 2008  
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```
=> s L6
    1 FILES SEARCHED...
    4 FILES SEARCHED...
    9 FILES SEARCHED...
   10 FILES SEARCHED...
L7      54 L6
```

```
=> dup rem L7
DUPLICATE IS NOT AVAILABLE IN 'DGENE'.
ANSWERS FROM THESE FILES WILL BE CONSIDERED UNIQUE
PROCESSING COMPLETED FOR L7
L8      48 DUP REM L7 (6 DUPLICATES REMOVED)
```

```
=> s L8 not py>2004
L9      12 L8 NOT PY>2004
```

```
=> d L9 ibib abs 1-12
```

```
L9      ANSWER 1 OF 12  DGENE  COPYRIGHT 2008 THE THOMSON CORP ON STN
ACCESSION NUMBER: AAZ36224  DNA          DGENE
TITLE:           Isolated nucleic acids encoding nitrile hydratase and amidase
                  from thermophilic Bacillus, useful for conversion of
```

acrylonitrile to acrylamide -  
INVENTOR: Oriol P J; Padmakumar R; Kim S H  
PATENT ASSIGNEE: (UNMS)UNIV MICHIGAN STATE.  
PATENT INFO: WO 9955719 A1 19991104 71  
APPLICATION INFO: WO 1999-US6888 19990330  
PRIORITY INFO: US 1998-83485 19980429  
US 1999-248528 19990210  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: 2000-013413 [01]  
DESCRIPTION: The 16S rRNA gene sequence for Bacillus sp. BR449.

AN AAZ36224 DNA DGENE  
AB The present sequence represents the 16S ribosomal (rRNA) gene sequence of Bacillus sp. BR449 (ATCC 202119). The genus/species of BR449 was determined by comparing its 16S rRNA gene sequence with that of other bacteria. A high level of identity was seen with other Bacillus sp., indicating that BR449 is a Bacillus. The specification describes a BR449 nitrile hydratase comprising an alpha subunit and a beta subunit, that is optimally active at greater than 55 degrees Celsius, and stable at greater than 60 degrees Celsius. The enzyme contains cobalt, and converts nitriles to amides without significant production of its corresponding acid. As the BR449 nitrile hydratase, unlike known nitrile hydratases, does not require a low temperature, cooling is not necessary and both reaction rate and product solubility are improved. The enzyme also has high resistance to substrate inhibition, allowing a high concentration of acrylonitrile in the reaction mixture. The nitrile hydratase and cells that express it, are used to convert acrylonitrile to acrylamide, a starting material for polymers, and may also be used to hydrate many other nitriles. The enzymatic production of acrylamide from acrylonitrile generates fewer waste products and requires less energy than the conventional copper-catalysed process. An associated amidase is used to convert amides to the corresponding acid. The nitrile hydratase polynucleotide is used to produce transformants for recombinant production of the nitrile hydratase without expression of the associated amidase.

L9 ANSWER 2 OF 12 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 2005-01712 BIOTECHDS <<LOGINID::20080205>>  
TITLE: Novel Comamonas testosteroni derived polynucleotide encoding alpha and beta subunits of nitrile hydratase enzyme, accessory protein, and amidase, useful for catalyzing hydration of nitriles to amides and amides to carboxylic acids;

isolation of nitrile-hydratase, an accessory protein and an amidase from Comamonas testosteroni useful as a biocatalyst for the hydration of a nitrile  
AUTHOR: PAYNE M S; DICOSIMO R; GAVAGAN J E; FALLON R D  
PATENT ASSIGNEE: PAYNE M S; DICOSIMO R; GAVAGAN J E; FALLON R D  
PATENT INFO: US 2004225116 11 Nov 2004  
APPLICATION INFO: US 2003-431966 8 May 2003  
PRIORITY INFO: US 2003-431966 8 May 2003; US 2003-431966 8 May 2003  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 2004-821018 [81]  
AN 2005-01712 BIOTECHDS <<LOGINID::20080205>>

AB DERWENT ABSTRACT:  
NOVELTY - An isolated polynucleotide (I) encoding the alpha, and beta subunits of a nitrile hydratase (NHase) enzyme, an accessory protein, and an amidase (Am) and comprising a fully defined Comamonas

testosteroni 5-MGAM-4D derived sequence (S1) of 3449 base pairs as given in the specification, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) an isolated polynucleotide (II) encoding a polypeptide comprising the alpha-subunit of NHase enzyme having fully defined C. testosteroni 5-MGAM-4D derived sequence (S2) of 210 amino acids as given in the specification, where (II) has fully defined sequence (S3) of 633 base pairs as given in the specification; (2) an isolated polynucleotide (III) encoding a polypeptide having 90% identity to (II); (3) an isolated polypeptide (IV) having (S2); (4) an isolated polynucleotide (V) encoding a polypeptide comprising the beta-subunit of NHase enzyme having a fully defined sequence (S4) of 218 amino acids as given in the specification, where (V) has a fully defined sequence (S5) of 657 base pairs as given in the specification; (5) an isolated polynucleotide (VI) encoding a polypeptide having 80% identity to (S4); (6) a polypeptide (VII) having (S4); (7) an isolated polynucleotide (VIII) encoding the alpha and beta subunits of NHase enzyme and having fully defined sequence of 1386 base pairs as given in the specification; (8) an isolated polynucleotide (IX) encoding the alpha and beta subunits of NHase enzyme and an accessory protein, and having a fully defined sequence of 2223 base pairs as given in the specification; (9) an isolated polynucleotide (X) encoding a polypeptide comprising an amidase enzyme having a fully defined sequence (S6) of 468 amino acids as given in the specification, where has a fully defined sequence of 1407 base pairs as given in the specification; (10) an isolated polynucleotide (XI) encoding a polypeptide having amidase enzyme and having 95% identity to polypeptide having (S6); (11) a polypeptide (XII) having (S6); (12) an isolated polynucleotide (XIII) encoding a polypeptide comprising an accessory protein and having a fully defined sequence (S7) of 71 amino acids as given in the specification, where (XIII) has a fully defined sequence of 216 base pairs as given in the specification; (13) a polypeptide (XIV) having (S7); (14) an expression vector (V1) comprising (II), (III), (VI), (VII), (VIII), (IX), (X), (XI) or (XIII); (15) an expression vector (V2) as contained in *Escherichia coli* SW132 designated ATCC PTA-5073 or as contained in *E. coli* SW137 designated ATCC PTA-5074; (16) transformed microbial host cell (TC1) comprising (V1), (V2) or (V3); (17) a purified transformed microbial host cell (TC2) chosen from *E. coli* SW132 designated ATCC PTA-5073 and a purified microbial host cell *E. coli* SW137 designated ATCC PTA-5074; (18) converting (M1) a substrate containing one or more nitrile functional groups to an amide, involves contacting, under suitable conditions, a transformed microbial host cell expressing a NHase polypeptide encoded by (IX) with a substrate containing one or more nitrile functional groups, and recovering the produced amide; (19) hydrating (M2) methacrylonitrile to methoacrylamide, involves contacting methacrylonitrile, under suitable reaction conditions, with a catalyst having NHase activity from *Comamonas testosteroni* 5-MGAM-4D; and (20) hydrating (M3) acrylonitrile to acrylamide, involves contacting acrylonitrile, under suitable reaction conditions, with a catalyst having NHase activity from *Comamonas testosteroni* 5-MGAM-4D.

BIOTECHNOLOGY - Preferred Microbial Host: TC1 is a bacterium, yeast, or filamentous fungi. TC1 is a bacterium chosen from *E. coli*, *Pseudomonas*, *Rhodococcus*, *Acinetobacter*, *Bacillus*, and *Streptomyces*, a yeast chosen from *Pichia*, *Hansenula* and *Saccharomyces* or a filamentous fungi chosen from *Aspergillus*, *Neurospora*, and *Penicillium*. TC1 is preferably *E. coli*. Preferred Method: In (M1), the substrate containing at least one nitrile functional group is a nitrile of formula  $R-C\equiv N$  (F1) or  $N=C-R-C\equiv N$  (F2). R = 1-9C alkyl, linear, branched, or cyclic optionally substituted, 1-9C alkenyl, linear, branched, or cyclic optionally substituted or 1-9C aryl, optionally substituted. The nitrile is 2-hydroxynitrile, 3-hydroxynitrile, or 4-hydroxynitrile. The R of (F2) is 1-4C alkyl,



linear, or branched. The nitrile is chosen from malononitrile, adiponitrile, glutaronitrile, and 2-methylglutaronitrile. The R of (F1) is 1-4C alkenyl, linear, or branched. The nitrile is preferably acrylonitrile or methacrylonitrile. In (M1)-(M3), the catalyst is in the form of whole cells, permeabilized microbial cells, one or more components of a microbial cell extract, partially purified enzyme, or purified enzyme. The catalyst is immobilized on or in a soluble or insoluble support. The catalyst is immobilized in alginate or carageenan.

USE - TC1 is useful for producing polypeptides, which involves culturing TC1 under suitable conditions and recovering the produced polypeptide (claimed). (I) is useful for catalyzing hydration of certain nitriles to corresponding amides and the amides to corresponding carboxylic acids.

EXAMPLE - *Comamonas testosteroni* 5-MGAM-4D (ATCC 55744) was grown in LB media at 37degreesC, with shaking. Genomic DNA was prepared. Southern analysis was performed on EcoRI restricted genomic DNA using *Pseudomonas putida* NRRL-18668 genes encoding nitrile hydratase alpha, and beta subunits as probe. The alpha and beta probes each showed positive hybridization to the same 5.7 kb EcoRI DNA fragment. Genomic DNA fragment encoding C.testosteroni 5-MGAM-4D NHase was cloned. The nucleotide sequence of the pKP57 insert was determined using an ABI 377-XL DNA sequencer. Nucleotide sequences of the pKP57 insert encoding NHase alpha, and beta-subunits were a fully defined sequence of 633 and 657 base pairs as given in the specification, respectively. Deduced amino acid sequences of the pKP57 insert for the alpha, and beta-subunits were a fully defined sequence of 210 and 218 amino acids as given in the specification, respectively. C.testosteroni 5-MGAM-4D NHase was produced by expressing the nucleotide. Production of alpha (23 kDa) and beta (23 kDa) proteins was confirmed by standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Growth and induction of *Escherichia coli* BL21 (DE3) cells harboring pSW131 was carried out. Cells were then harvested by centrifugation, washed twice in buffer (0.1 M potassium phosphate pH 7.0) and suspended at 100 mg wet cells/ml in buffer. The nitrilase activity assay mix included cells (50 mg/ml), 3-hydroxy- valeronitrile (0.3 M) and buffer (0.1 M potassium phosphate, pH 7.0) stirred at ambient temperature. High performance liquid chromatography (HPLC) analysis demonstrated 17 % conversion of 3-HVN to the corresponding amide (3-hydroxyvaleramide) in 15 minutes. Genomic DNA from C.testosteroni 5-MGAM-4D was prepared, restricted with PstI, and subjected to Southern analysis using a standard PCR product comprising the first 0.6 kb of the pKP57 insert as a probe. Probe labeling, hybridization and detection systems. This probe gave hybridized to a 2.4 kb PstI fragment. Genomic DNA digested with PstI was subjected to standard agarose gel electrophoresis. DNA fragments in the size range of approximately 2-4 kb were isolated and ligated into PstI restricted pUC1 g. This plasmid library was plated and screened with the same 0.6 kb probe. Probe labeling, hybridization and detection were done using ECL random primer labeling and detection systems. A positively hybridizing colony was isolated and determined to contain an insert of 2.4 kb (pKP59). Nucleotide sequencing confirmed that the insert is a DNA fragment that overlaps the EcoRI DNA fragment previously cloned (pKP57). Thus, by combining the nucleotide sequences from pKP57 and pKP59, the complete nucleotide sequence for the amidase gene was determined (a fully defined sequence of 1407 base pairs as given in the specification). The deduced amidase amino acid sequence was a fully defined sequence of 468 amino acids as given in the specification. The nucleotide sequence of a 7.4 kb DNA fragment from C.testosteroni 5-MGAM-4D comprising complete coding sequences for amidase and NHase comprises a fully defined sequence of 7415 base pairs as given in the specification. (37 pages)

L9 ANSWER 3 OF 12 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 2002-02697 BIOTECHDS <<LOGINID::20080205>>  
TITLE: Culturing microbes which produce nitrile-hydratase with  
keto-sugar or sugar alcohol and cobalt to increase yield;  
with use of Rhodococcus rhodochrous culture medium

AUTHOR: Ryuno K; Kobayashi E  
PATENT ASSIGNEE: Mitsubishi-Rayon  
LOCATION: Tokyo, Japan.  
PATENT INFO: WO 2001/0936 27 Sep 2001  
APPLICATION INFO: WO 2001-JP2232 21 Mar 2001  
PRIORITY INFO: JP 2000-78484 21 Mar 2000  
DOCUMENT TYPE: Patent  
LANGUAGE: Japanese  
OTHER SOURCE: WPI: 2001-656855 [75]

AN 2002-02697 BIOTECHDS <<LOGINID::20080205>>  
AB Method of culturing a microbe which can produce a  
nitrile-hydratase (EC-4.2.1.84) uses a culture medium which contains a  
sugar alcohol and/or a keto sugar, and cobalt ion. The enzyme  
is used as an energy-saving catalyst in the production of amides from  
nitriles, especially acrylamide from acrylonitrile.  
The presence of a sugar component such as fructose or mannitol reduces  
the growth inhibition due to the cobalt ion and gives a high yield of  
microbial cells with nitrile-hydratase activity in a short time. In an  
example, Rhodococcus rhodochrous was cultured and nitrile-hydratase  
activity was measured. (18pp)

L9 ANSWER 4 OF 12 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 1995-14739 BIOTECHDS <<LOGINID::20080205>>  
TITLE: Bioconversion of acrylonitrile to acrylamide in aqueous  
two-phase system;  
using Pseudomonas putida with nitrile-hydratase activity

AUTHOR: Zhao F; Wu J; Liao H  
CORPORATE SOURCE: Univ.Shanghai-Jiao-Tong  
LOCATION: Department of Biological Science and Technology, Shanghai  
Jiao Tong University, Shanghai 200030, People's Republic of  
China.

SOURCE: Ind.Microbiol.; (1995) 25, 3, 6-12  
CODEN: GOWEEK

DOCUMENT TYPE: Journal  
LANGUAGE: Chinese  
AN 1995-14739 BIOTECHDS <<LOGINID::20080205>>

AB Acrylamide was prepared from acrylonitrile in aqueous  
two-phase system using Pseudomonas putida JP-1 cells containing  
nitrile-hydratase (EC-4.2.1.84) as biocatalyst. The aqueous two-phase system  
comprised PEG 6,000 (0.05 g/ml). K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O (0.20 g/ml),  
acrylonitrile (0.30 mol/l) and wet cells (0.10 g/ml). The pH of  
the system was 9.0 and the optimum temperature for the conversion was  
determined to be 25 deg. At pH 10.0, nitrile-hydratase activity  
in P. putida JP-1 cells was at its most stable. The lower the temperature the  
better the thermostability of the nitrile-hydratase in the cells. During  
the enzyme-catalyzed conversion, acrylonitrile was  
added at certain time intervals to produce acrylamide  
and the acrylamide formed was purified. (4 ref)

L9 ANSWER 5 OF 12 USP2 on STN  
ACCESSION NUMBER: 2003:213842 USP2 <<LOGINID::20080205>>  
TITLE: Method for producing methacrylic acid acrylic acid with  
a combination of enzyme catalysts  
INVENTOR(S): Dicosimo, Robert, Rockland, DE, United States  
Fallon, Robert D., Elkton, MD, United States

PATENT ASSIGNEE(S): Gavagan, John E., Wilmington, DE, United States  
Manzer, Leo Ernest, Wilmington, DE, United States  
E. I. du Pont de Nemours and Company, Wilmington, DE,  
United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6670158	B2	20031230
APPLICATION INFO.:	US 2002-67652		20020205 (10)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	GRANTED		
PRIMARY EXAMINER:	Lilling, Herbert J.		
NUMBER OF CLAIMS:	6		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	0 Drawing Figure(s); 0 Drawing Page(s)		
LINE COUNT:	626		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides a process for the hydrolysis of acrylonitrile to acrylic acid, and for the hydrolysis of methacrylonitrile to methacrylic acid, in high yield and at high concentration with high specificity. Acrylonitrile or methacrylonitrile is hydrolyzed in a suitable aqueous reaction mixture by a catalyst characterized by a nitrile hydratase and amidase activity of Comamonas testosteroni 5-MGAM-4D, producing the corresponding acid. The acrylic acid or methacrylic acid is isolated as the acid or corresponding salt.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 6 OF 12 USPAT2 on STN

ACCESSION NUMBER: 2002:32221 USPAT2 <<LOGINID::20080205>>  
TITLE: Method for stabilizing nitrilase activity and  
preserving microbial cells with carbamate salts  
INVENTOR(S): Dicosimo, Robert, Rockland, DE, United States  
Ben-Bassat, Arie, Newark, DE, United States  
Fallon, Robert D., Elkton, MD, United States  
PATENT ASSIGNEE(S): E. I. du Pont de Nemours and Company, Wilmington, DE,  
United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6677149	B2	20040113
APPLICATION INFO.:	US 2001-854498		20010514 (9)
RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. US 2000-614914, filed on 12 Jul 2000, now patented, Pat. No. US 6368804 Continuation-in-part of Ser. No. US 1999-352015, filed on 12 Jul 1999, now patented, Pat. No. US 6251646		
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	GRANTED		
PRIMARY EXAMINER:	Marx, Irene		
NUMBER OF CLAIMS:	14		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	0 Drawing Figure(s); 0 Drawing Page(s)		
LINE COUNT:	1029		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method for preserving immobilized or unimmobilized microbial cells having nitrilase activity and for stabilizing the nitrilase activity of unimmobilized or immobilized microbial cells has been developed. Aqueous suspensions containing at least 100 mM bicarbonate, carbonate, or carbamate salts limit microbial contamination of the stored enzyme catalyst, as well as stabilize the desired nitrilase activity of the unimmobilized or immobilized cells. Microorganisms which are

characterized by an nitrilase activity and are stabilized and preserved by this method include Acidovorax facilis 72-PF-15 (ATCC 55747), Acidovorax facilis 72-PF-17 (ATCC 55745), Acidovorax facilis 72W (ATCC 55746), and transformed microbial cells having nitrilase activity, the host cells transformed with Acidovorax facilis 72W nitrilase activity. Especially preferred is an embodiment using ammonium carbamate as the inorganic salt.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 7 OF 12 WPIDS COPYRIGHT 2008 THE THOMSON CORP on STN  
 ACCESSION NUMBER: 1988-201154 [29] WPIDS  
 DOC. NO. CPI: C1988-089709 [21]  
 TITLE: Production of amide cpds. from corresp. nitrile cpd. - using water soluble enzyme comprising 2 heterogeneous sub-units as catalyst  
 DERWENT CLASS: D16; E19  
 INVENTOR: GOMI K; KAWAKAMI K; NAGANO O  
 PATENT ASSIGNEE: (KEIS-N) KEISITSU RYUBUN SHI  
 COUNTRY COUNT: 1

PATENT INFO ABBR.:

PATENT NO	KIND DATE	WEEK	LA	PG	MAIN IPC
JP 63137688	A 19880609	(198829)*	JA	7[0]	
JP 03054558	B 19910820	(199137)	JA		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
JP 63137688 A		JP 1986-284150	19861201
JP 03054558 B		JP 1986-284150	19861201

PRIORITY APPLN. INFO: JP 1986-284150 19861201

AN 1988-201154 [29] WPIDS

AB JP 63137688 A UPAB: 20050428

In the production of amide cpds., an amide cpd. is generated from the corresp. nitrile cpd. by using a water-soluble enzyme comprising two heterogeneous subunits as a catalyst.

Specifically, the enzyme is derived from Rhodococcus sp. AK-32 (FERM P-8269). The enzyme is purified from the culture of the strain by homogenising cells, precipitation with ammonium sulphate, dialysis, anion-exchange, gel filtration, etc.. Crude enzyme solution is used, but amidase activity in the enzyme solution must be removed. The reaction conditions are pH 8-9, at 0-10 deg.C, 0.01-0.1 mole ion/l, and nitrile concentration 0.1-5 weight%. Pref. nitrile cpds. used contain less

than

6C.Methacrylonitrile and acrylonitrile are most pref.. Methacrylamide and acrylamide are produced as a result.

ADVANTAGE - The amide cpds. are produced in high yield, rapidly under mild condition, and with a small amount of catalyst and without generation of side prods.. - In an example, the enzyme derived from Rhodococcus sp. AK-32 was dissolved in 0.05M KH2PO4 (pH 8.5) solution (100 pts.) at 0.5 deg.C. Concentration of the enzyme was 0.01 weight%.

Methacrylonitrile

(17 pts.) was added at 0.5 deg.C. After 2 hours, methacrylonitrile was consumed completely and methacrylamide crystal was produced. The crystal was separated from the reaction solution and was washed with water. No

methacrylic acid was detected in the reaction solution.

L9 ANSWER 8 OF 12 USPATOLD on STN  
ACCESSION NUMBER: 1973:72176 USPATOLD  
TITLE: PASTE FOR GUMMED TAPE AND PROCESS FOR PRODUCING THE  
SAME FROM HYDROLYZED STARCH  
INVENTOR(S): YOSHIZAWA A  
KITAZAWA T  
PATENT ASSIGNEE(S): NIHON RIKI SEISHI KABUSHIKI KAISHA

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 3770672	A	19731106
APPLICATION INFO.:	US 1971-114342		19710201

	NUMBER	DATE
PRIORITY INFORMATION:	US 1971-114342	19710210
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	GRANTED	
PRIMARY EXAMINER:	LEE, LESTER L	
LINE COUNT:	431	
CAS INDEXING IS AVAILABLE FOR THIS PATENT.		
CAS INDEXING IS AVAILABLE FOR THIS PATENT.		

L9 ANSWER 9 OF 12 USPATOLD on STN  
ACCESSION NUMBER: 1972:73888 USPATOLD  
TITLE: MACROPOROUS ENZYME REACTOR  
INVENTOR(S): REYNOLDS JOHN H  
PATENT ASSIGNEE(S): MONSANTO COMPANY, INC.

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 3705084	A	19721205
APPLICATION INFO.:	US 1971-112802		19710201

	NUMBER	DATE
PRIORITY INFORMATION:	US 1970-20639	19700318
	US 1971-112802	19710204
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	GRANTED	
PRIMARY EXAMINER:	GOLIAN, JOSEPH M	
LINE COUNT:	564	
CAS INDEXING IS AVAILABLE FOR THIS PATENT.		
CAS INDEXING IS AVAILABLE FOR THIS PATENT.		

L9 ANSWER 10 OF 12 USPATOLD on STN  
ACCESSION NUMBER: 1967:12877 USPATOLD  
TITLE: Compositions and method for binding bile acids in vivo  
including hypocholesteremics  
INVENTOR(S): TENNENT DAVID M  
WOLF FRANK J

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 3308020	A	19670307
APPLICATION INFO.:	US 1961-139880		19610922

	NUMBER	DATE

PRIORITY INFORMATION: US 1961-139880 19610922  
DOCUMENT TYPE: Utility  
FILE SEGMENT: GRANTED  
PRIMARY EXAMINER: MEYERS, ALBERT T  
LINE COUNT: 518  
CAS INDEXING IS AVAILABLE FOR THIS PATENT.  
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 11 OF 12 BIOENG COPYRIGHT 2008 CSA on STN  
ACCESSION NUMBER: 2004011015 BIOENG <<LOGINID::20080205>>  
DOCUMENT NUMBER: 356263  
TITLES: Acrylamide production in an ultrafiltration-membrane  
bioreactor using cells of *Brevibacterium imperialis* CBS  
489-74  
AUTHOR: Cantarella, M; Spera, A; Cantarella, L; Alfani, F  
CORPORATE SOURCE: Univ of L'Aquila, L'Aquila, Italy  
SOURCE: Journal of Membrane Science. Vol. 147, no. 2, pp.  
279-290. 2 Sep 1998.  
Published by: ELSEVIER SCI B.V., AMSTERDAM, (NETHERLANDS)  
ISSN: 0376-7388  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AN 2004011015 BIOENG <<LOGINID::20080205>>  
AB Both differential and integral UF-membrane reactors were tested for the  
bioconversion of acrylonitrile into acrylamide. Use  
was made of the commercially available flat membrane cell Amicon Mod.52  
and the UF-membranes FS81PP, GR81PP, and YM100. The enzymatic  
reaction was catalyzed by the nitrile hydratase (NHase) present in  
resting cells of *Brevibacterium imperialis* CBS 489-74. The system was  
operated at 4 degree C and 10 degree C. Acrylonitrile  
concentration ranged from 50 to 500 mM. The membrane resistance to  
chemicals was complete at acrylonitrile and acrylamide  
concentrations up to 800 mM and 2 M, respectively. No rejection of solute  
was determined. Membranes totally retained the resting cells  
and no fouling was observed working with 2 and 16 mg of biocatalyst in  
stirred systems. Membrane compaction was apparently responsible for  
roughly 35% flux loss during the first 3-4 h of operation. The laboratory  
scale membrane bioreactor, continuously operating, allowed to show the  
dependence of enzyme deactivation on acrylonitrile  
concentration and process time. Substrate concentration higher than 100  
mM were highly detrimental for NHase stability. The acrylamide  
yield reached in the multi-cycle process operating with 5.6 g/l of  
resting cells was 93.7% and the product concentration during  
roughly 450 h of bioconversion attained 8.3% (w/v). Decay of specific  
membrane flux was 98% of the initial value.

L9 ANSWER 12 OF 12 Elsevier BIOBASE COPYRIGHT 2008 Elsevier Science B.V.  
on STN  
ACCESSION NUMBER: 1999265887 ESBIOBASE <<LOGINID::20080205>>  
TITLE: Role of cytochrome P450 2E1 in the metabolism of  
acrylamide and acrylonitrile in mice  
AUTHOR: Sumner S.C.J.; Fennell T.R.; Moore T.A.; Chanas B.;  
Gonzalez F.; Ghanayem B.I.  
CORPORATE SOURCE: S.C.J. Sumner, Chem. Industry Inst. of Toxicology, 6  
Davis Dr., Res. Triangle Park, NC 27709-2137, United  
States.  
E-mail: Sumner@ciit.org  
SOURCE: Chemical Research in Toxicology, (1999), 12/11  
(1110-1116), 37 reference(s)  
CODEN: CRTOEC ISSN: 0893-228X  
DOCUMENT TYPE: Journal; Article

COUNTRY: United States  
LANGUAGE: English  
SUMMARY LANGUAGE: English

AB Acrylonitrile (AN) and acrylamide (AM) are commonly used in the synthesis of plastics and polymers. In rodents, AM and AN are metabolized to the epoxides glycidamide and cyanoethylene oxide, respectively. The aim of this study was to determine the role of cytochrome P450 in the metabolism of AM and AN in vivo. Wild-type (WT) mice, WT mice pretreated with aminobenzotriazole (ABT, 50 mg/kg ip, 2 h pre-exposure), and mice devoid of cytochrome P450 2E1 (P450 2E1-null) were treated with 50 mg/kg [.sup.1.sup.3CLAM po. WT mice and P450 2E1-null mice were treated with 2.5 or 10 mg/kg [.sup.1.sup.3CLAN po. Urine was collected for 24 h, and metabolites were characterized using .sup.1.sup.3C NMR. WT mice excreted metabolites derived from the epoxides and from direct GSH conjugation with AM or AN. Only metabolites derived from direct GSH conjugation with AM or AN were observed in the urine from ABT-pretreated WT mice and P450 2E1-null mice. On the basis of evaluation of urinary metabolites at these doses, these data suggest that P450 2E1 is possibly the only cytochrome P450 enzyme involved in the metabolism of AM and AN in mice, that inhibiting total P450 activity does not result in new pathways of non- P450 metabolism of AM, and that mice devoid of P450 2E1 do not excrete metabolites of AM or AN that would be produced by oxidation by other cytochrome P450s. P450 2E1-null mice may be an appropriate model for the investigation of the role of oxidative metabolism in the toxicity or carcinogenicity of these compounds.

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(FILE 'HOME' ENTERED AT 11:39:14 ON 05 FEB 2008)

FILE 'REGISTRY' ENTERED AT 11:39:29 ON 05 FEB 2008

L1 1 SEA ABB=ON PLU=ON 107-13-1/RN  
L2 1 SEA ABB=ON PLU=ON 79-06-1/RN

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS, CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB, DRUGMONOG2, DRUGU, EMBAL, EMBASE, ...' ENTERED AT 11:40:16 ON 05 FEB 2008

FILE 'REGISTRY' ENTERED AT 11:40:35 ON 05 FEB 2008

SEL L1 CHEM  
SEL L2 CHEM

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS, CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB, DRUGMONOG2, DRUGU, EMBAL, EMBASE, ...' ENTERED AT 11:40:50 ON 05 FEB 2008

SEA E1-17 (S) E18-26

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16 FILE AGRICOLA  
9 FILE ANABSTR  
19 FILE ANTE  
5 FILE AQUALINE  
2 FILE AQUASCI  
42 FILE BIOENG  
107 FILE BIOSIS  
143 FILE BIOTECHABS  
143 FILE BIOTECHDS  
29 FILE BIOTECHNO

16 FILE CABA  
 3865 FILE CAPLUS  
 64 FILE CEABA-VTB  
 30 FILE CIN  
 4 FILE CONFSCI  
 2 FILE CROPU  
 3 FILE DDFB  
 4 FILE DDFU  
 51 FILE DGENE  
 10 FILE DISSABS  
 3 FILE DRUGB  
 4 FILE DRUGU  
 1 FILE EMBAL  
 64 FILE EMBASE  
 43 FILE ESBIODASE  
 7 FILE FROSTI  
 14 FILE FSTA  
 6 FILE HEALSARE  
 3317 FILE IFIPAT  
 57 FILE LIFESCI  
 53 FILE MEDLINE  
 45 FILE NTIS  
 291 FILE PASCAL  
 2 FILE PHIN  
 191 FILE PROMT  
 43 FILE RDISCLOSURE  
 306 FILE SCISEARCH  
 1 FILE SYNTHLINE  
 240 FILE TOXCENTER  
 72 FILE USGENE  
 22634 FILE USPATFULL  
 3144 FILE USPATOLD  
 2942 FILE USPAT2  
 10 FILE WATER  
 4551 FILE WPIDS  
 28 FILE WPIFV  
 4551 FILE WPINDEX

L3 QUE ABB=ON PLU=ON (ACRYLON/BI OR ACRYLONITRILE/BI OR  
 CARBACRYL/BI OR CYANOETHENE/BI OR CYANOETHYLENE/BI OR FUMIGRAIN  
 /BI OR "NSC 6362"/BI OR PROPENENITRILE/BI OR VCN/BI OR  
 VENTOX/BI OR "VINYL CYANIDE"/BI OR 107-13-1/BI OR 2-PROPENENITRILE/BI OR 29754-21-0/BI OR 63908-52-1/BI OR 769126-92-3/BI OR 769134-66-9/BI) (S) (ACRYLAMIDE/BI OR "ACRYLIC AMIDE"/BI OR "BIO-ACRYLAMIDE 50"/BI OR ETHYLENECARBOXAMIDE/BI OR "NSC 7785"/BI OR PROPENAMIDE/BI OR "VINYL AMIDE"/BI OR 2-PROPENAMIDE/BI OR 79-06-1/BI)

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 SEA L3 (S) ENZYME#####  
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 12 FILE BIOTECHNO  
 1 FILE CABA  
 47 FILE CAPLUS  
 12 FILE CEABA-VTB  
 2 FILE CIN



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30 FILE DGENE
5 FILE EMBASE
11 FILE ESBIODBASE
1 FILE FROSTI
8 FILE FSTA
1 FILE HEALSAFE
16 FILE IFIPAT
24 FILE LIFESCI
3 FILE MEDLINE
13 FILE PASCAL
8 FILE PROMT
1 FILE RDISCLOSURE
7 FILE SCISEARCH
3 FILE TOXCENTER
72 FILE USGENE
125 FILE USPATFULL
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13 FILE USPAT2
1 FILE WATER
54 FILE WPIDS
1 FILE WPIFV
54 FILE WPINDEX

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L4

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QUE ABB=ON PLU=ON L3 (S) ENZYM#####
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SEA L4 (S) (CONVERT### OR PRODUC### OR GENERAT###)
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1 FILE ANABSTR
13 FILE BIOENG
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45 FILE BIOTECHDS
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15 FILE CAPLUS
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27 FILE DGENE
1 FILE EMBASE
5 FILE ESBIODBASE
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7 FILE PASCAL
4 FILE PROMT
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75 FILE USPATFULL
3 FILE USPATOLD
10 FILE USPAT2
15 FILE WPIDS
15 FILE WPINDEX

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L5

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QUE ABB=ON PLU=ON L4 (S) (CONVERT### OR PRODUC### OR
GENERAT###)
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SEA L5 AND (DETECT### OR DETERMIN### OR MEASUR### OR QUANTITAT#
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1 FILE BIOENG
9 FILE BIOTECHABS
9 FILE BIOTECHDS

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1      FILE ESBIOBASE
1      FILE LIFESCI
1      FILE PASCAL
72     FILE USPATFULL
3      FILE USPATOLD
9      FILE USPAT2
6      FILE WPIDS
6      FILE WPINDEX
L6     QUE ABB=ON  PLU=ON  L5 AND (DETECT### OR DETERMIN### OR
      MEASUR### OR QUANTITAT###)
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      D RANK

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BIOTECHNO, ESBIOBASE, LIFESCI' ENTERED AT 11:53:34 ON 05 FEB 2008
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L9     12 SEA ABB=ON  PLU=ON  L8 NOT PY>2004
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